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Capillary red blood cell velocimetry by phase-resolved optical coherence tomography

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Abstract

We present a phase-resolved optical coherence tomography (OCT) method to extend Doppler OCT for the accurate measurement of the red blood cell (RBC) velocity in cerebral capillaries. OCT data were acquired with an M-mode scanning strategy (repeated A-scans) to account for the single-file passage of RBCs in a capillary, which were then high-pass filtered to remove the stationary component of the signal to ensure an accurate measurement of phase shift of flowing RBCs. The angular frequency of the signal from flowing RBCs was then quantified from the dynamic component of the signal and used to calculate the axial speed of flowing RBCs in capillaries. We validated our measurement by RBC passage velocimetry using the signal magnitude of the same OCT time series data.

Cerebral blood flow plays an important role in regulating nutrients and oxygen that support brain function. Capillary hemodynamics is of particular importance as it is where the nutrients and oxygen are predominantly exchanged with local brain tissue. However, the quantitative measurement of blood flow velocity in capillaries is challenging due to their small size (around 5–10 μm) and the discontinuity and single-file feature of RBCs flowing in a capillary [1–3]. Utilizing the advantages of noninvasive, fast volumetric imaging at micrometer-scale resolution without exogenous contrast agents, optical coherence tomography (OCT) [4,5] has been widely used to measure the structural and functional features of microvascular networks. OCT angiography [6–9] has emerged as a very useful imaging technique for visualizing perfused microvasculature networks, but it is not able to provide quantitative blood flow velocity information. Doppler OCT is capable of the quantitative volumetric measurement of axial blood flow speed by detecting the Doppler frequency shift of backscattered light from a moving particle (e.g., a flowing RBC) [10]. Compared to the spectrogram-based method that uses short-time fast Fourier transformation or wavelet transformation [10], the phase-resolved method that measures the phase change between sequential A-lines to reconstruct axial speed has become the method of choice for

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Doppler OCT as it possesses higher velocity sensitivity and is compatible with Fourier domain OCT [11]. Doppler OCT can accurately measure blood flow speed in large vessels but has had challenges measuring RBC speed in capillaries [10,12–14] where RBCs flow slowly, discontinuously, and in a single file. In addition, an OCT imaging voxel is usually mixed with stationary and dynamic components when detecting blood flow in a capillary, which also makes it challenging for standard phase-resolved Doppler OCT to accurately quantify RBC speed in a capillary.

To apply OCT for RBC speed measurements in capillaries, Srinivasan *et al.* [1] showed that the power spectrum bandwidth of the autocorrelation function of the OCT signal correlated with the blood flow speed in capillaries, but this method has not yet provided measures of absolute blood flow speed. Ren *et al.* [15] exploited a method similar to that used by multiphoton microscopy (MPM) to estimate RBC speed by detecting the RBC Doppler phase transient induced by the passage of an RBC through a capillary. However, similar to the MPM method, this method is usually limited to one position or a single vessel and is incapable of estimating speed in large vessels with dense RBCs flowing through. Choi *et al.* [2] proposed an OCT speckle signal decorrelation rate-based method to estimate the RBC speed in capillaries. However, the influence of hematocrit in the decorrelation rate makes it challenging for this method to get reliable RBC speed measurements. In addition, phantom calibration was required for this method, raising the complexity of the measurement. Dynamic light scattering optical coherence tomography (DLS-OCT) [16,17] has shown the potential for measuring total blood flow speed in large vessels, but more investigation is needed to realize robust capillary velocimetry.

In this work, we propose a reliable method to extend phase-resolved Doppler OCT to accurately measure RBC axial speed in both large vessels and capillaries. This method is based upon the first, to the best of our knowledge, *in vivo* observation that a continuous OCT phase shift can be detected from a single RBC moving in a capillary. To the best of our knowledge, no phase-resolved Doppler OCT technique has used this well-behaved signal to obtain accurate RBC axial speed in capillaries (<10 μm in size). We validated our measurement of RBC total speed with the RBC passage velocimetry method using the OCT signal magnitude of the same OCT time series data.

A spectral domain OCT system (Thorlabs Inc.) [18] employing a large-spectral-bandwidth near-infrared light source (center wavelength: 1310 nm; bandwidth: 170 nm) was used for this study. The axial resolution of the OCT system was 3.5 μm and the lateral resolution was 3.5 μm as well when using a 10 $\times$ objective (NA = 0.26) for brain tissue imaging. The OCT system can image as deep as 900 μm from the brain surface. The A-line sampling rate was 47,000 A-lines/s.

Figure 1(A) illustrates the OCT detection of an RBC flowing in a capillary. In principle, the movement of the RBC in the axial direction will result in a phase shift of the OCT signal from which we can accurately determine the axial speed of the RBC with Eq. (1),

$$v_z = \frac{\omega_0}{2nk_0}, \quad (1)$$

where $\omega_0 = d\phi/dt$ is the angular frequency of phase shift, $n = 1.35$ is the refractive index of tissue, and k_0 is the center wavenumber of the source spectrum ($k_0 = 4.8 \mu\text{m}^{-1}$ for our OCT system).

The challenge is to correctly extract the phase shift information of the moving RBC despite confounding effects. First, to account for the discontinuity of individual RBCs flowing through capillaries, we applied an M-mode scanning strategy with A-scans repeated for ~ 16 ms (i.e., $n_t = 800$ individual A-scans) at each scanning position, as illustrated in Fig. 1(B). This was to ensure that individual RBCs could be measured continuously as they moved through the OCT measurement voxel, but we do investigate below the effectiveness of the method if fewer repeated A-scans are obtained at each position. Second, as the capillary size ($5\text{--}10 \mu\text{m}$) is comparable to the OCT resolution size ($3.5 \times 3.5 \times 3.5 \mu\text{m}$), the OCT signal in each voxel was usually composed of stationary and dynamic signal components. The presence of the stationary signal confounds the quantification of the phase changes due to RBC movement. To remove the stationary signal component [19], we applied a high-pass filter [Fig. 1(C), cutoff frequency = 200 Hz with an equivalent cutoff speed of $v_{z,\text{low}} = 0.0967 \text{ mm/s}$] to the repeated A-scans. The resultant phase of the dynamic component was thus largely enhanced to give a more reliable phase shift, as illustrated by the red lines in the second and third rows of Fig. 2.

We obtained the angular frequency of the Doppler shift from the slope of the unwrapped phase signal as directly calculating the phase wrap frequency from the second row of Fig. 2 proved to be noisy. As illustrated by the bottom row of Fig. 2, we estimated the average angular frequency based on the histogram of the unwrapped phase slope $\omega_i = d\phi_i/dt$ using Eq. (2),

$$\omega_0 = \frac{\sum_{i=1}^M (N_i \cdot \omega_i)}{\sum_{i=1}^M N_i}, \quad (2)$$

where N_i is the number of slopes falling in the ω_i bin (bin width = 0.1 rad/s, corresponding to a speed bin width of 0.0077 mm/s), M is the number of ω_i having $N_i > N_{\text{max}}/2$, and N_{max} is the maximum value of N_i . In addition, we used an angular frequency threshold $|\omega_i| > \omega_{\text{thold}} = |\phi_1 - \phi_{n_t}|/2 \cdot (n_t \cdot dt)$ to avoid the bias effect of $\omega_i = 0$ as it is likely that, within the acquisition time of the M-scan, only a small portion of the signal detected an RBC moving within the capillary. Without this threshold, the nonflow signal ($\omega_i = 0$) would dominate the phase slope histogram. With the threshold $|\omega_i| > \omega_{\text{thold}}$, the portions of the data time series having a moving RBC is readily identified and the mean RBC speed could be correctly determined. Further, the directional information of the movement can also be determined

according to the sign of the calculated phase change. For the three examples in Fig. 2, we measured the axial speeds of 0.55, 1.44, and -0.94 mm/s, respectively.

In addition, we note that the RBC passage speed in some capillaries could also be estimated based on measuring the time for an RBC to move through the OCT voxel as estimated from the OCT signal magnitude [the top rows of Figs. 2(A) and 2(B)] and assuming the diameter of the RBC (we assumed a diameter of $5\text{ }\mu\text{m}$) [15,20]. We thus used this RBC passage speed to verify our measurement of RBC axial speed using the capillary angle to convert between axial and total speeds. It is worth mentioning that OCT-based RBC passage velocimetry can only be applied to a few vessels that clearly reveal a magnitude variation when an RBC flows through, such as the examples shown in Figs. 2(A) and 2(B). Indeed, it is not possible to estimate speed in the majority vessels where a flowing RBC cannot be identified, as shown in Fig. 2(C). In addition, due to the phase oscillation, it is difficult to locate RBC passages automatically. Therefore, RBC passages are manually identified—a laborious task—and then used to calculate RBC passage speed. Figure 1(D) summarizes the flow chart of the proposed capillary velocimetry using phase-resolved OCT.

Three CD1 mice were used in this study to validate our method. A 3×3 mm cranial window was created over the somatosensory cortex and the exposed brain was covered with agarose gel and a glass coverslip. Isoflurane anesthetization was applied during surgery and during data acquisition. The imaging field of view (FOV) was set to be $300\text{ }\mu\text{m} \times 300\text{ }\mu\text{m}$, and the total acquired A-lines for each FOV was $800 \times 200 \times 200$ ($n_t \times n_x \times n_y$), which took ~ 11.6 min. All animal experimental procedures were reviewed and approved by the Massachusetts General Hospital Subcommittee on Research Animal Care.

Comparing the OCT angiogram maximum intensity projection (MIP) and v_z MIP in Figs. 3(A) and 3(B), we note that the proposed method can measure RBC axial speed in most capillaries (67 out of 84 vessel segments). To verify the measured RBC speed, we compared the measured total speed ($v_z/\cos(\theta)$, where θ is the angle of the vessel to the optical axis that is estimated with an OCT angiogram) with the RBC passage speed (assuming an RBC diameter of $5\text{ }\mu\text{m}$) from 50 randomly selected locations where RBC passage could be identified. As shown in Fig. 3(C), the speed measured by the RBC-induced phase shifts were correlated with the speed measured by the RBC passage, and the regression line had a slope of 1.08 (with the intercept forced to 0), indicating similar quantitative values for the RBC speed. The difference from the expected slope of 1 is likely a result of an error in the assumed diameter of the RBC and in the estimation of the vessel angle. We also studied the effect of the number of A-line repetitions (n_t) on the RBC speed measurement. Qualitatively, as shown in Fig. 3(D), we see that the larger the n_t (longer repeated acquisition time), the better the quality of the RBC axial speed image. Particularly, the measurements in capillaries with slow RBC speeds were greatly improved with increased repetitions. We also calculated the ratio of the number of nonzero v_z pixels for different A-line repetitions versus that of 800 A-line repetitions to quantitatively assess the impact of a reduced number of A-line repetitions, as shown in Fig. 3(E). In our opinion, $n_t = 50$ A-line repetitions ($t_{\text{rpt}} = 1.05$ ms for each A-line location) provides an acceptable trade-off between measurement duration and image quality. This corresponds to a total acquisition time of ~ 45 s for a volumetric 3D capillary RBC speed measurement spanning 200×200 pixels in the lateral dimensions. To

test the depth imaging ability of the proposed technique, we focused at three different depths to acquire three volumetric images and then combined them together to form the axial speed image across the whole cerebral cortex layer. In Fig. 3(F), it is seen that the RBC axial speed in large vessels and small capillaries was successfully detected across the whole cortex. The reason for low/no signal beyond the depth of $\sim 800\ \mu\text{m}$ is due to the lower optical signal retrieved from the white matter beneath the cerebral cortex.

Comparing the M-mode (i.e., repeat A-scans) scanning approach for capillary velocimetry to previously reported approaches with repeated B-scans [1,2,15], the M-mode scanning strategy provides a faster sampling of the phase shift such that RBC axial speeds up to 11 mm/s can be measured accurately (with an A-scan rate of 47 kHz measured at 1310 nm center wavelength). The sampling rate for repeated B-scans is typically 100 times slower, limiting accurate measurement of the RBC axial speed to approximately 0.11 mm/s. Given that RBC speeds of 0.5–1.0 mm/s are typical in capillaries [21], the advantage of repeated A-scans for accurate capillary velocimetry is clear. The M-mode scanning approach was also applied by Meemon and Rolland [14] and Shi *et al.* [13] to enhance phase-resolved Doppler OCT of multiple velocity ranges. Compared to their approaches of skipping a different number of A-scans at each position to calculate the phase changes with different time intervals for multiple velocity ranges, the proposed method utilized all A-scans to obtain a continuous phase shift with which a wide range of blood flow speeds (0.1–11 mm/s) could be determined with a single calculation. The use of the high-pass filter further makes the proposed method more appropriate for capillary velocimetry. In addition, the proposed phase-slope-histogram-based method to determine the angular frequency proved to be robust and less sensitive to phase noise, which has affected Doppler OCT for *in vivo* blood flow speed measurements [11,13,14].

The OCT signal magnitude-based RBC passage velocimetry method could be used to estimate the flow speed in some vessels. However, it is worth noting that (1) the estimate was only possible in a small fraction of capillaries with the RBC passage velocimetry method, (2) it is difficult to identify RBC passage automatically based on the OCT signal, and (3) there can easily be errors in the assumed RBC diameter. As an example, to prepare Fig. 3(C) in the Letter, we examined about 500 voxels in which we could calculate RBC speed using phase-resolved measurements and found that RBC speed could be calculated in only 50 of the voxels using the RBC passage velocimetry method. In addition, it took us ~ 2 h for this RBC passage calculation of the 50 voxels compared to a total of ~ 5 min for the phase-resolved calculation of the whole volume ($200 \times 200 \times 100$ voxels). Similar to MPM strip-pattern-based capillary velocimetry [3], accurate RBC speed could be measured based on the OCT intensity stripe patterns by consecutively repeating B-scans over a cross-sectional plane (z - x) [20]. However, this method is usually limited to small transverse capillaries that only allow a single RBC passing through, and an advanced image processing algorithm has to be utilized to determine the strip angle. Although the proposed phase-resolved-based OCT velocimetry method cannot measure the transverse flow speed, it is capable of accurately quantifying the RBC speed in the majority of capillaries, as demonstrated in Fig. 3. Indeed, we note that we could more reliably measure RBC speed in more vessels (including large vessels and capillaries) using the proposed method than we could measure using the RBC passage velocimetry. In addition, the proposed data processing

method is robust and automatic, significantly simplifying volumetric blood flow speed measurements. Further, it is worth mentioning that compared to MPM and other layer-scanning-based optical capillary velocimetry methods [22], the proposed method is much faster for volumetric imaging as only a 2D XY scan is required for 3D imaging.

In this work, we determined the vessel angle by calculating the angle between vessel centers of consecutive Z planes. This method may introduce errors in calculating total speed, however, as demonstrated by Fig. 3(C). The calculated total speed has a good linear relationship with the estimated RBC passage speed, indicating that the accuracy of vessel angle determination is sufficient for this study. The exploration of accurate estimation of vessel angle [23–25] is beyond the scope of this Letter. In the future, more efforts are needed to more accurately calculate the vessel angle in order to quantify the blood flow total speed. Although the acquisition time for a 3D volume containing $800 \times 200 \times 200$ ($n_t \times n_x \times n_y$) A-lines took ~ 11.6 min, the acquisition time for each position only took ~ 17.6 ms, which was short enough to avoid motion artifacts primarily resulting from cardiac pulses (71–194 ms [26]) and respiration (261–750 ms [27]). In addition, $n_t = 50$ A-line repetitions ($t_{\text{pt}} = 1.05$ ms for each A-line location) provides an acceptable trade-off between measurement duration and image quality, and the effect from motion artifacts at a specific measurement position are even more negligible.

In summary, we introduced a phase-resolved Doppler OCT method to accurately measure RBC velocity in both large vessels and capillaries. The automatic data processing method greatly simplifies volumetric RBC flow speed measurements. This proposed method of capillary velocimetry proved to be a robust method of mapping capillary RBC speeds across the microvascular network.

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References

1. Srinivasan VJ, Radhakrishnan H, Lo EH, Mandeville ET, Jiang JY, Barry S, Cable AE. Biomed Opt Express. 2012; 3:612. [PubMed: 22435106]
2. Choi WJ, Li Y, Qin W, Wang RK. Biomed Opt Express. 2016; 7:4859. [PubMed: 28018711]
3. Kleinfeld D, Mitra PP, Helmchen F, Denk W. Proc Natl Acad Sci USA. 1998; 95:15741. [PubMed: 9861040]
4. Huang D, Swanson EA, Lin CP, Schuman JS, Stinson WG, Chang W, Hee MR, Flotte T, Gregory K, Puliafito CA. Science. 1991; 254:1178. [PubMed: 1957169]
5. Drexler W, Fujimoto, JG. Optical Coherence Tomography: Technology and Applications. Springer; 2015.
6. Wang RK, Jacques SL, Ma Z, Hurst S, Hanson SR, Gruber A. Opt Express. 2007; 15:4083. [PubMed: 19532651]
7. Srinivasan VJ, Jiang JY, Yaseen MA, Radhakrishnan H, Wu W, Barry S, Cable AE, Boas DA. Opt Lett. 2010; 35:43. [PubMed: 20664667]
8. Makita S, Hong Y, Yamanari M, Yatagai T, Yasuno Y. Opt Express. 2006; 14:7821. [PubMed: 19529151]
9. Wei W, Xu J, Baran U, Song S, Qin W, Qi X, Wang RK. J Biomed Opt. 2016; 21:036005.

10. Chen Z, Milner TE, Dave D, Nelson JS. *Opt Lett.* 1997; 22:64. [PubMed: 18183104]
11. Zhao Y, Chen Z, Saxer C, Xiang S, de Boer JF, Nelson JS. *Opt Lett.* 2000; 25:114. [PubMed: 18059800]
12. Leitgeb RA, Werkmeister RM, Blatter C, Schmetterer L. *Prog Retin Eye Res.* 2014; 41:26. [PubMed: 24704352]
13. Shi L, Qin J, Reif R, Wang RK. *J Biomed Opt.* 2013; 18:106015. [PubMed: 24165741]
14. Meemon P, Rolland JP. *Biomed Opt Express.* 2010; 1:955. [PubMed: 21258521]
15. Ren H, Du C, Park K, Volkow ND, Pan Y. *Appl Phys Lett.* 2012; 100:233702. [PubMed: 22904572]
16. Lee J, Wu W, Jiang JY, Zhu B, Boas DA. *Opt Express.* 2012; 20:22262. [PubMed: 23037374]
17. Tang J, Erdener SE, Li B, Fu B, Sakadzic S, Carp SA, Lee J, Boas DA. *J Biophoton.* 2017; online. doi: 10.1002/jbio.201700070
18. Lee J, Srinivasan V, Radhakrishnan H, Boas DA. *Opt Express.* 2011; 19:21258. [PubMed: 22108978]
19. Srinivasan VJ, Sakadži S, Gorczynska I, Ruvinskaya S, Wu W, Fujimoto JG, Boas DA. *Opt Express.* 2010; 18:2477. [PubMed: 20174075]
20. Lee J, Wu W, Lesage F, Boas DA. *J Cereb Blood Flow Metab.* 2013; 33:1707. [PubMed: 24022621]
21. Kamoun WS, Chae SS, Lacorre DA, Tyrrell JA, Mitre M, Gillissen MA, Fukumura D, Jain RK, Munn LL. *Nat Methods.* 2010; 7:655. [PubMed: 20581828]
22. Guevara-Torres A, Joseph A, Schallek J. *Biomed Opt Express.* 2016; 7:4228. [PubMed: 27867728]
23. Trasischker W, Werkmeister RM, Zotter S, Baumann B, Torzicky T, Pircher M, Hitzenberger CK. *J Biomed Opt.* 2013; 18:116010. [PubMed: 24247747]
24. Wang Y, Bower BA, Izatt JA, Tan O, Huang D. *J Biomed Opt.* 2007; 12:041215. [PubMed: 17867804]
25. Singh AS, Kolbitsch C, Schmoll T, Leitgeb RA. *Biomed Opt Express.* 2010; 1:1047. [PubMed: 21258529]
26. Ho, D., Zhao, X., Gao, S., Hong, C., Vatner, DE., Vatner, SF. *Current Protocols in Mouse Biology.* Wiley; 2011. p. 123-139.
27. Zehendner CM, Luhmann HJ, Yang JW. *PLoS ONE.* 2013; 8:e62628. [PubMed: 23658756]

Fig. 1.

(A) OCT detection of an RBC flowing in a capillary. (B) M-mode scanning strategy. (C) Top: the high-pass filter kernel; bottom: frequency response of the designed high-pass filter. (D) Flow chart illustrates the proposed phase-resolved OCT method for capillary velocimetry.

Fig. 2.

Three representative examples showing the OCT detection of RBC speed, the enhancement of the phase shift signal with a high-pass filter, and the procedure for calculating the angular frequency of the Doppler shift. First row: OCT signal magnitude; second row: OCT phase signal; third row: unwrapped phase signal; and fourth row: histogram of the phase slope (ω_i). Blue lines: OCT raw data; red lines: with high-pass filter. RBC passages are manually identified and marked with green shadows in the first row.

Fig. 3.

(A) OCT angiogram MIP spanning a depth of 170–330 μm from the cortical surface. (B) v_z MIP maps over the same depth range as (A). (C) Comparison between the measured axial speed ($v_z/\cos(\theta)$, where θ is the angle of the vessel) and the RBC passage speed (v_{passage}) from 50 randomly selected locations. (D) Results from mouse #2 showing the improvement with an increasing number of repeated A-scans (n_t) on capillary velocimetry ($t_{\text{rpt}} = 0.42, 1.05, 2.1, 4.1, \text{ and } 16.8$ ms, corresponding to $n_t = 20, 50, 100, 200, \text{ and } 800$, respectively); *en face* X–Y v_z MIP maps were obtained over the depth range of 100–265 μm from the cortical surface. (E) Ratio of the number of v_z pixels for different A-line repetitions divided by that of $n_t = 800$. (F) X–Z MIP (~100 μm stack along Y) of axial speed across the whole cerebral cortex obtained from mouse #3. Color bar: axial blood flow speed (mm/s). Positive value: blood flows toward the brain surface; negative value: blood flows into the brain. No spatial filtering was applied to the results.